

ID NO: 1 under conditions where, on a Southern blot, said probe will identify single 3.25 kb BamHI fragments from *M. bovis* BCG and *M. tuberculosis* H37Rv DNA, but will not hybridize with BamHI-digested DNA from either *M. smegmatis* or *M. vaccae*--

### Remarks

Claims 1-23 were pending and are canceled in the instant application. Claims 53-70 are added. Previously, claims 24-52 were added during prosecution and claim 50 was allowed. Subject matter from previous claim 50 is found in present claim 70. The present claims have been amended to recite a method of detecting the presence of antibodies to *M. bovis* and *M. tuberculosis* in a biological sample, support for which can be found in previously filed claims 25-50 and in the specification, particularly in example I on page 16 through page 20, line 3, and on page 30, line 9-16 and in figure 3.

### CONCLUSION

On the basis of the amendments and remarks presented herein, Applicants believe that this application is now in condition for immediate allowance. Applicants respectfully request that the Examiner pass this application to issue, and an early notice of such is requested. The Examiner is invited to call the undersigned attorneys for discussion of any outstanding issues.

Respectfully submitted,

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## VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 1-23 have been cancelled.

Claims 53-70 have been added as follows:

53. (New) A method of detecting the presence of antibodies to *M. bovis* and *M. tuberculosis* in a biological sample, said method comprising:  
combining said sample with a protein having the amino acid sequence of SEQ ID NO:2 or an antigenic determinant thereof; and  
detecting antibodies bound to said protein.
54. (New) The method of Claim 53, wherein said protein is immobilized on a solid support.
55. (New) The method of Claim 54, wherein said solid support is nitrocellulose.
56. (New) .The method of Claim 53, wherein said sample comprises one or more of sputum, blood, and serum.
57. (New) The method of Claim 53, wherein said detecting is by a qualitative detection system.
58. (New) The method of Claim 57, wherein said qualitative detection system is a horseradish peroxidase-protein A detection system.
59. (New) The method of Claim 53, wherein said detecting is by a quantitative detection system.
60. (New) The method of Claim 59, wherein said quantitative detection system is a radioimmunoassay.
61. (New) The method of Claim 53, further comprising:

combining a control biological sample with said protein; and  
comparing the detection of said binding to the binding of antibodies in the control sample  
with said protein.

62. (New) A method of detecting the presence of *M. bovis* and *M. tuberculosis* in a  
biological sample, said method comprising;  
a) lysing the cells in said sample;  
b) combining said lysate with antibodies to a protein having the amino acid  
sequence of SEQ ID NO:2 or an antigenic determinant thereof; and  
c) detecting said antibodies bound to protein in said lysate.

63. (New) The method of Claim 62, wherein said lysate is immobilized on a solid  
support.

64. (New) The method of Claim 63, wherein said solid support is nitrocellulose.

65. (New) The method of Claim 62, wherein said detecting is by a qualitative detection  
system.

66. (New) The method of Claim 65, wherein said qualitative detection system is a  
horseradish peroxidase-protein A detection system.

67. (New) The method of Claim 62, wherein said detecting is by a quantitative detection  
system.

68. (New) The method of Claim 67, wherein said quantitative detection system is a  
radioimmunoassay.

69. (New) The method of Claim 62, further comprising:  
culturing a diagnostic sample to produce colonies of bacteria present therein, whereby said  
culture represents said biological sample.

70. (New) A method of detecting the presence of antibodies to a virulent Mycobacterium in a biological sample, said method comprising:  
combining said sample with a purified protein of a mycobacterium other than *M. bovis* BCG, wherein said protein is a homolog of the protein of SEQ ID NO:2; is an immunogenic membrane-associated protein of said mycobacterium; and is encoded by DNA which is capable of hybridizing with a DNA probe having the complete sequence represented in SEQ ID NO: 1 under conditions where, on a Southern blot, said probe will identify single 3.25 kb BamHI fragments from *M. bovis* BCG and *M. tuberculosis* H37Rv DNA, but will not hybridize with BamHI-digested DNA from either *M. smegmatis* or *M. vaccae*--

## PENDING CLAIMS

53. A method of detecting the presence of antibodies to *M. bovis* and *M. tuberculosis* in a biological sample, said method comprising:  
combining said sample with a protein having the amino acid sequence of SEQ ID NO:2 or an antigenic determinant thereof; and  
detecting antibodies bound to said protein.
54. The method of Claim 53, wherein said protein is immobilized on a solid support.
55. The method of Claim 54, wherein said solid support is nitrocellulose.
56. The method of Claim 53, wherein said sample comprises one or more of sputum, blood, and serum.
57. The method of Claim 53, wherein said detecting is by a qualitative detection system.
58. The method of Claim 57, wherein said qualitative detection system is a horseradish peroxidase-protein A detection system.
59. The method of Claim 53, wherein said detecting is by a quantitative detection system.
60. The method of Claim 59, wherein said quantitative detection system is a radioimmunoassay.
61. The method of Claim 53, further comprising:  
combining a control biological sample with said protein; and  
comparing the detection of said binding to the binding of antibodies in the control sample with said protein.

62. A method of detecting the presence of *M. bovis* and *M. tuberculosis* in a biological sample, said method comprising;
- a) lysing the cells in said sample;
  - b) combining said lysate with antibodies to a protein having the amino acid sequence of SEQ ID NO:2 or an antigenic determinant thereof; and
  - c) detecting said antibodies bound to protein in said lysate.
63. The method of Claim 62, wherein said lysate is immobilized on a solid support.
64. The method of Claim 63, wherein said solid support is nitrocellulose.
65. The method of Claim 62, wherein said detecting is by a qualitative detection system.
66. The method of Claim 65, wherein said qualitative detection system is a horseradish peroxidase-protein A detection system.
67. The method of Claim 62, wherein said detecting is by a quantitative detection system.
68. The method of Claim 67, wherein said quantitative detection system is a radioimmunoassay.
69. The method of Claim 62, further comprising:  
culturing a diagnostic sample to produce colonies of bacteria present therein, whereby said culture represents said biological sample.
70. A method of detecting the presence of antibodies to a virulent Mycobacterium in a biological sample, said method comprising:  
combining said sample with a purified protein of a mycobacterium other than *M. bovis* BCG, wherein said protein is a homolog of the protein of SEQ ID NO:2; is an immunogenic membrane-associated protein of said mycobacterium; and is encoded by DNA which is capable of hybridizing with a DNA probe having the complete sequence represented in SEQ

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